



## SEQUENCE LISTING

<110> YEASTERN BIOTECH CO., LTD

<120> A FUNGAL IMMUNOMODULATORY PROTEIN (FIP) PREPARED BY MICROORGANISMS AND USES THEREOF

<130> PCT/CN2004/001044

<140> US 10/572,563

<141> 2006-03-17

<150> PCT/CN2004/001044

<151> 2004-09-14

<150> US 60/503,547

<151> 2003-09-17

<160> 15

<170> PatentIn version 3.3

<210> 1

<211> 336

<212> DNA

<213> Ganoderma lucidium

<220>

<221> variation

<222> (1)..(336)

<223> improved DNA sequence of G. lucidium FIP for expressing in yeast, FIP-yeast

<400> 1

atgtctgata ctgctttgat tttcagattg gcttggatg ttaagaagtt gtctttcgat 60

tacactccaa actggggtag aggtAACCCa aacaacttca ttgatactgt tactttccca 120

aaggtttga ctgataaggc ttacacttac agagttgctg tttctggtag aaacttgggt 180

gttaagccat cttacgctgt tgaatctgat ggttctcaaa aggttaactt ctggaaatac 240

aactctggtt acggtattgc tgataactaac actattcaag ttttcgttgt tgatccagat 300

actaacaacg atttcattat tgctcaatgg aactga 336

<210> 2

<211> 336

<212> DNA

<213> Ganoderma lucidium

<220>

<221> gene

<222> (1)..(336)

<223> original FIP codon, FIP-1z

<400> 2  
atgtccgaca ctgccttgat cttcaggctc gcctgggacg tgaagaagct ctcgttcgac 60  
tacaccccgaa actggggccg cggcaacccc aacaacttca tcgacactgt caccttccc 120  
aaagtcttga ccgacaaggc gtacacgtac cgcgtcgccg tctccggacg gaacctcgac 180  
gtgaaaccct cgtacgcggt cgagagcgac ggctcgaga aggtcaactt cctcgagtac 240  
aactccgggt atggcatagc ggacacgaac acgatccagg tggcggttgcgac 300  
accaacaacg acttcatcat cgcccaactgg aactag 336

<210> 3

<211> 40

<212> DNA

<213> Artificial

<220>

<223> primer

<220>  
<221> primer  
<222> (1)..(40)  
<223> forward primer of FIP-yeast

<400> 3

aaaaaaaaaa ggatcccgca atgtctgata ctgcttgat 40

<210> 4  
<211> 41  
<212> DNA  
<213> Artificial

<220>

<223> primer

<220>  
<221> primer  
<222> (1)..(41)  
<223> reverse primer of FIP-yeast

<400> 4

aaaaaaaaaa acacgtgtca actagtttagt tccattgagc a 41

<210> 5  
<211> 41  
<212> DNA  
<213> Artificial

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<220>
<223> primer
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<220>
<221> primer
<222> (1)..(41)
<223> forward primer of FIP-lz

<400> 5
aaaaaaaaaa ggatcccgca atgtccgaca ctgccttgat c 41

<210> 6
<211> 42
<212> DNA
<213> Artificial

<220>
<223> primer

<220>
<221> primer
<222> (1)..(42)
<223> reverse primer of FIP-lz

<400> 6
aaaaaaaaaa acacgtgtca actagttgt tccctagttc ca 42

<210> 7
<211> 111
<212> PRT
<213> Ganoderma lucidium

<220>
<221> PEPTIDE
<222> (1)..(111)
<223> FIP amino acid sequence

<400> 7

Met Ser Asp Thr Ala Leu Ile Phe Arg Leu Ala Trp Asp Val Lys Lys
1 5 10 15

Leu Ser Phe Asp Tyr Thr Pro Asn Trp Gly Arg Gly Asn Pro Asn Asn
20 25 30

Phe Ile Asp Thr Val Thr Phe Pro Lys Val Leu Thr Asp Lys Ala Tyr
35 40 45

Thr Tyr Arg Val Ala Val Ser Gly Arg Asn Leu Gly Val Lys Pro Ser

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50

55

60

Tyr Ala Val Glu Ser Asp Gly Ser Gln Lys Val Asn Phe Leu Glu Tyr  
65 70 75 80

Asn Ser Gly Tyr Gly Ile Ala Asp Thr Asn Thr Ile Gln Val Phe Val  
85 90 95

Val Asp Pro Asp Thr Asn Asn Asp Phe Ile Ile Ala Gln Trp Asn  
100 105 110

<210> 8  
<211> 111  
<212> PRT  
<213> Ganoderma tsugae

<220>  
<221> PEPTIDE  
<222> (1)..(111)  
<223> FIP amino acid sequence

<400> 8

Met Ser Asp Thr Ala Leu Ile Phe Arg Leu Ala Trp Asp Val Lys Lys  
1 5 10 15

Leu Ser Phe Asp Tyr Thr Pro Asn Trp Gly Arg Gly Asn Pro Asn Asn  
20 25 30

Phe Ile Asp Thr Val Thr Phe Pro Lys Val Leu Thr Asp Lys Ala Tyr  
35 40 45

Thr Tyr Arg Val Ala Val Ser Gly Arg Asn Leu Gly Val Lys Pro Ser  
50 55 60

Tyr Ala Val Glu Ser Asp Gly Ser Gln Lys Val Asn Phe Leu Glu Tyr  
65 70 75 80

Asn Ser Gly Tyr Gly Ile Ala Asp Thr Asn Thr Ile Gln Val Phe Val  
85 90 95

Val Asp Pro Asp Thr Asn Asn Asp Phe Ile Ile Ala Gln Trp Asn  
100 105 110

<210> 9

<211> 113  
<212> PRT  
<213> *Flammulina velutipes*

<220>  
<221> PEPTIDE  
<222> (1)..(113)  
<223> FIP amino acid sequence

<400> 9

Ser Ala Thr Ser Leu Thr Phe Gln Leu Ala Tyr Leu Val Lys Lys Ile  
1 5 10 15

Asp Phe Asp Tyr Thr Pro Asn Trp Gly Arg Gly Thr Pro Ser Ser Tyr  
20 25 30

Ile Asp Asn Leu Thr Phe Pro Lys Val Leu Thr Asp Lys Lys Tyr Ser  
35 40 45

Tyr Arg Val Val Val Asn Gly Ser Asp Leu Gly Val Glu Ser Asn Phe  
50 55 60

Ala Val Thr Pro Ser Gly Gly Gln Thr Ile Asn Phe Leu Gln Tyr Asn  
65 70 75 80

Lys Gly Tyr Gly Val Ala Asp Thr Lys Thr Ile Gln Val Phe Val Val  
85 90 95

Pro Asp Thr Gly Asn Ser Glu Glu Tyr Ile Ile Ala Glu Trp Lys Lys  
100 105 110

Thr

<210> 10  
<211> 49  
<212> DNA  
<213> Artificial

<220>  
<223> primer

<220>  
<221> primer  
<222> (1)..(49)  
<223> forward primer of FIP

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<400> 10
aaaaaactcga gaaaagagag gctgaagcta tgtccgacac tgccttgat 49

<210> 11
<211> 31
<212> DNA
<213> Artificial

<220>
<223> primer

<220>
<221> primer
<222> (1)..(31)
<223> reverse primer of FIP

<400> 11
aaaaaacacgt gtcaactagt tagttccatt g 31

<210> 12
<211> 315
<212> DNA
<213> Artificial

<220>
<223> recombinant DNA sequence for encoding recombinant protein

<220>
<221> CDS
<222> (22)..(315)
<223> recombinant protein containing alpha-factor and partial FIP

<400> 12
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          Met Arg Phe Pro Ser Ile Phe Thr Ala Val
          1           5           10

tta ttc gca gca tcc tcc gca tta gct gct cca gtc aac act aca aca
Leu Phe Ala Ala Ser Ser Ala Leu Ala Pro Val Asn Thr Thr 99
          15          20          25

gaa gat gaa acg gca caa att ccg gct gaa gct gtc atc ggt tac tca
Glu Asp Glu Thr Ala Gln Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser
          30          35          40

gat tta gaa ggg gat ttc gat gtt gct gtt ttg cca ttt tcc aac agc 147
Asp Leu Glu Gly Asp Phe Asp Val Ala Val Leu Pro Phe Ser Asn Ser
          45          50          55

aca aat aac ggg tta ttg ttt ata aat act act att gcc agc att gct 195
Thr Asn Asn Gly Leu Leu Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala
          60          65          70

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gct aaa gaa gaa ggg gta tct ctc gag aaa aga gag gct gaa gct atg 291  
Ala Lys Glu Glu Gly Val Ser Leu Glu Lys Arg Glu Ala Glu Ala Met  
75 80 85 90

tcc gac act gcc ttg atc ttc agg 315  
Ser Asp Thr Ala Leu Ile Phe Arg  
95

<210> 13  
<211> 98  
<212> PRT  
<213> Artificial

<220>  
<223> Synthetic Construct

<400> 13

Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser  
1 5 10 15

Ala Leu Ala Ala Pro Val Asn Thr Thr Glu Asp Glu Thr Ala Gln  
20 25 30

Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser Asp Leu Glu Gly Asp Phe  
35 40 45

Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu  
50 55 60

Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val  
65 70 75 80

Ser Leu Glu Lys Arg Glu Ala Glu Ala Met Ser Asp Thr Ala Leu Ile  
85 90 95

Phe Arg

<210> 14  
<211> 624  
<212> DNA  
<213> Artificial  
  
<220>  
<223> recombinant sequence

<220>

<221> CDS  
 <222> (22)..(624)  
 <223> recombinant sequence comprising alpha-factor and FIP

<400> 14

cggtacccgg ggatccaaac g	atg	aga	ttt	cct	tca	att	ttt	act	gca	gtt	51					
	Met	Arg	Phe	Pro	Ser	Ile	Phe	Thr	Ala	Val						
1						5				10						
tta	ttc	gca	gca	tcc	tcc	gca	tta	gct	gct	cca	aac	act	aca	aca	99	
Leu	Phe	Ala	Ala	Ser	Ser	Ala	Leu	Ala	Ala	Pro	Val	Asn	Thr	Thr		
15						20						25				
gaa	gat	gaa	acg	gca	caa	att	ccg	gct	gaa	gct	gtc	atc	ggt	tac	tca	147
Glu	Asp	Glu	Thr	Ala	Gln	Ile	Pro	Ala	Glu	Ala	Val	Ile	Gly	Tyr	Ser	
30						35						40				
gat	tta	gaa	ggg	gat	ttc	gat	gtt	gct	ttg	cca	ttt	tcc	aac	agc	195	
Asp	Leu	Glu	Gly	Asp	Phe	Asp	Val	Ala	Val	Leu	Pro	Phe	Ser	Asn	Ser	
45						50						55				
aca	aat	aac	ggg	tta	ttg	ttt	ata	aat	act	act	att	gcc	agc	att	gct	243
Thr	Asn	Asn	Gly	Leu	Leu	Phe	Ile	Asn	Thr	Thr	Ile	Ala	Ser	Ile	Ala	
60						65					70					
gct	aaa	gaa	gaa	ggg	gta	tct	ctc	gag	aaa	aga	gag	gct	gaa	gct	atg	291
Ala	Lys	Glu	Glu	Gly	Val	Ser	Leu	Glu	Lys	Arg	Glu	Ala	Glu	Ala	Met	
75						80				85		90				
tct	gat	act	gct	ttg	att	ttc	aga	ttg	gct	tgg	gat	gtt	aag	aag	ttg	339
Ser	Asp	Thr	Ala	Leu	Ile	Phe	Arg	Leu	Ala	Trp	Asp	Val	Lys	Lys	Leu	
95						100						105				
tct	ttc	gat	tac	act	cca	aac	tgg	ggt	aga	ggt	aac	cca	aac	aac	ttc	387
Ser	Phe	Asp	Tyr	Thr	Pro	Asn	Trp	Gly	Arg	Gly	Asn	Pro	Asn	Asn	Phe	
110						115					120					
att	gat	act	ttt	cca	aag	gtt	ttg	act	gat	aag	gct	tac	act			435
Ile	Asp	Thr	Val	Thr	Phe	Pro	Lys	Val	Leu	Thr	Asp	Lys	Ala	Tyr	Thr	
125						130					135					
tac	aga	gtt	gct	tct	ggt	aga	aac	ttg	ggt	gtt	aag	cca	tct	tac		483
Tyr	Arg	Val	Ala	Val	Ser	Gly	Arg	Asn	Leu	Gly	Val	Lys	Pro	Ser	Tyr	
140						145					150					
gct	gtt	gaa	tct	gat	ggt	tct	caa	aag	gtt	aac	ttc	ttg	gaa	tac	aac	531
Ala	Val	Glu	Ser	Asp	Gly	Ser	Gln	Lys	Val	Asn	Phe	Leu	Glu	Tyr	Asn	
155						160					165		170			
tct	ggt	tac	ggt	att	gct	gat	act	aac	act	att	caa	gtt	ttc	gtt	gtt	579
Ser	Gly	Tyr	Ile	Ala	Asp	Thr	Asn	Thr	Ile	Gln	Val	Phe	Val	Val		
175						180					185					
gat	cca	gat	act	aac	aac	gat	ttc	att	att	gct	caa	tgg	aac	tga		624
Asp	Pro	Asp	Thr	Asn	Asn	Asp	Phe	Ile	Ile	Ala	Gln	Trp	Asn			
190						195					200					

<210> 15  
<211> 200  
<212> PRT  
<213> Artificial

<220>  
<223> Synthetic Construct

<400> 15

Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser  
1 5 10 15

Ala Leu Ala Ala Pro Val Asn Thr Thr Glu Asp Glu Thr Ala Gln  
20 25 30

Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser Asp Leu Glu Gly Asp Phe  
35 40 45

Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu  
50 55 60

Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val  
65 70 75 80

Ser Leu Glu Lys Arg Glu Ala Glu Ala Met Ser Asp Thr Ala Leu Ile  
85 90 95

Phe Arg Leu Ala Trp Asp Val Lys Lys Leu Ser Phe Asp Tyr Thr Pro  
100 105 110

Asn Trp Gly Arg Gly Asn Pro Asn Asn Phe Ile Asp Thr Val Thr Phe  
115 120 125

Pro Lys Val Leu Thr Asp Lys Ala Tyr Thr Tyr Arg Val Ala Val Ser  
130 135 140

Gly Arg Asn Leu Gly Val Lys Pro Ser Tyr Ala Val Glu Ser Asp Gly  
145 150 155 160

Ser Gln Lys Val Asn Phe Leu Glu Tyr Asn Ser Gly Tyr Gly Ile Ala  
165 170 175

Asp Thr Asn Thr Ile Gln Val Phe Val Val Asp Pro Asp Thr Asn Asn  
180 185 190

Asp Phe Ile Ile Ala Gln Trp Asn  
195 200